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UTILITY PATENT APPLICATION TRANSMITTAL

(Only for new nonprovisional applications under 37 C.F.R. § 1.53(b))

Attorney Docket No.	OMRF 176
First Inventor or Application Identifier	Jordan J. N. Tang
Title	PROTEASE INHIBITORS THAT OVERCOME DRUG RESISTANCE
Express Mail Label No.	EL 320 554 585 US

JC525 U.S. PTO 09/506,994 02/18/00

APPLICATION ELEMENTS

See MPEP chapter 600 concerning utility patent application contents.

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1. [X] * Fee Transmittal Form *(e.g., PTO/SB/17)* *(Submit an original and a duplicate for fee processing)*
2. [X] Specification [*Total Pages* 20]
   (preferred arrangement set forth below)
   - Descriptive title of the Invention
   - Cross References to Related Applications
   - Statement Regarding Fed sponsored R & D
   - Reference to Microfiche Appendix
   - Background of the Invention
   - Brief Summary of the Invention
   - Brief Description of the Drawings *(if filed)*
   - Detailed Description
   - Claim(s)
   - Abstract of the Disclosure
3. [X] Drawing(s) *(35 U.S.C. 113)* [*Total Sheets* 4]
4. Oath or Declaration [*Total Pages* 3]
   a. [X] Unexecuted
   b. [ ] Copy from a prior application (37 C.F.R. § 1.63(d)) *(for continuation/divisional with Box 16 completed)*
      i. [ ] DELETION OF INVENTOR(S) Signed statement attached deleting inventor(s) named in the prior application, see 37 C.F.R. §§ 1.63(d)(2) and 1.33(b).

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5. [ ] Microfiche Computer Program *(Appendix)*
6. Nucleotide and/or Amino Acid Sequence Submission *(if applicable, all necessary)*
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ACCOMPANYING APPLICATION PARTS

7. [ ] Assignment Papers (cover sheet & document(s))
8. [ ] 37 C.F.R.§3.73(b) Statement *(when there is an assignee)* [ ] Power of Attorney
9. [ ] English Translation Document *(if applicable)*
10. [ ] Information Disclosure Statement (IDS)/PTO-1449 [ ] Copies of IDS Citations
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12. [X] Return Receipt Postcard (MPEP 503) *(Should be specifically itemized)*
13. [ ] * Small Entity Statement(s) *(PTO/SB/09-12)* [ ] Statement filed in prior application, Status still proper and desired
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Jordan J. N. Tang and Arun K.Ghosh

Serial No.: Express Mail Label No.: EL320554585US

Filed: February 18, 2000 Date of Deposit February 18, 2000

For: PROTEASE INHIBITORS THAT OVERCOME DRUG RESISTANCE

Assistant Commissioner
for Patents
Washington, D.C. 20231

EXPRESS MAIL TRANSMITTAL LETTER
FOR PATENT APPLICATION AND CERTIFICATE OF MAILING

Sir:

Pursuant to 35 U.S.C. § 21(a) as amended by Public Law 97-247 and 37 C.F.R. § 1.10, Jordan J. N. Tang and Arun K. Ghosh enclose for filing the attached patent application entitled "PROTEASE INHIBITORS THAT OVERCOME DRUG RESISTANCE", which claims priority to U.S.S.N. 60/120,835 filed February 19, 1999. The application includes 1 page of Abstract, 18 pages of specification, 1 page of claims, 4 sheets of informal drawings, and an unexecuted Declaration. An executed Declaration, Assignments to Oklahoma Medical Research Foundation and The Board of Trustees of the University of Illinois and Verified Statements Claiming Small Entity Status will be submitted shortly. A check in the amount of $345.00 to cover one half of the filing fee is enclosed.

The Commissioner is hereby authorized to charge our deposit order account no. 01-2507 in the amount of $345.00, which represents the difference between the filing fee for a large entity and small entity.

This application is being filed on February 18, 2000 by mailing the application to the Assistant Commissioner for Patents, Washington, D.C. 20231 via the United States Postal Service "Express Mail Post Office to Addressee" service under 37 C.F.R. § 1.10. The Express Mail label number appears in the heading of this paper which is attached to the application papers pursuant to 37 C.F.R. § 1.10(b).

The Commissioner is hereby authorized to charge any fees that may be required, or credit any overpayment to Deposit Order Account No. 01-2507. To facilitate this process, applicants have enclosed a duplicate of this letter.

All correspondence concerning this application should be mailed to:

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Respectfully submitted,

Patrea L. Pabst
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Date: February 18, 2000

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CERTIFICATE OF MAILING UNDER 37 C.F.R. § 1.10

I hereby certify that this Express Mail Transmittal Letter for Patent Application and any documents referred to as attached therein are being deposited with the United States Postal Service on this date, February 18, 2000, in an envelope as "Express Mail Post Office to Addressee" service under 37 C.F.R. § 1.10, mailing label number EL 320 554 585 US, addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.

Date: February 18, 2000

Jean Hicks

PROVISIONAL PATENT APPLICATION

BY

JORDAN J. N. TANG

AND

ARUN K. GHOSH

FOR

PROTEASE INHIBITORS THAT OVERCOME DRUG RESISTANCE

PROTEASE INHIBITORS THAT OVERCOME DRUG RESISTANCE

Background of the Invention

This application claims priority to U.S.S.N. 60/120,835 filed February 19, 1999 by Jordan J.N. Tang and Arun K. Ghosh.

This application is generally in the field of drugs to treat drug resistant pathogens, and in particular relates to protease inhibitors that do not elicit drug-resistant mutations in the pathogens they inhibit, such as the human immunodeficiency virus (HIV).

Drug resistance generally is a problem with the treatment of most pathogens, including bacteria and viruses. A variety of methods have been used, the most common being determining which drugs the pathogen is sensitive to, then treating the patient with a drug that the pathogen is sensitive to. Another approach is the use of a "cocktail", a mixture of two or three different drugs, preferably operating by different mechanisms of action, to block the life cycle of the pathogen before it can develop drug resistance. In the case of a virus such as HIV, this latter approach has been widely adopted, primarily through the use of one or two nucleoside drugs that inhibit replication by interacalation into the viral nucleic acid, in combination with a protease inhibitor that prevents replication. Unfortunately, even with the use of cocktails, HIV mutates extremely rapidly, and becomes resistant even to these combinations of drugs.

The HIV protease gene codes for a protease which, upon expression as part of the *gag-pol* protein, processes *gag* and *gag-pol* polyproteins into individual structural proteins and enzymes for the assembly of HIV virions (Debouck et al. (1987), *J. Med. Chem. Res.* 21:1-17). Mutation of the active-site residues of HIVPr renders the mutant virus non-infectious (Kohl et al. (1988), *Proc. Natl. Acad. Sci. USA* 85:4686-4690; Peng et al. (1989), *J. Virol.* 63:2550-2555), which established the HIVPr as a therapeutic target. As a result, many HIVPr inhibitors have been synthesized and tested (Wlodawer and Erickson (1993), *Ann. Rev. Biochem.* 62:843-855), among

which four have been marketed: saquinavir (Ro 31-8959, Craig et al. (1991), *Antiviral Res.* 16:295-305), indinavir (L-735,524, Dorsey et al. (1994), *J. Med. Chem.* 37:3443-3451), ritonivir (ABT-538, Kempf et al. (1995), *Proc. Natl. Acad. Sci. USA* 92:2484-2488) and nelfinevir (Patick et al. (1996), *Antimicrob. Agents Chemother.* 40:292-297). Structures are shown in Figures 1a-d. These drugs are among the most powerful compounds to suppress HIV replication, as demonstrated both in tissue culture and in clinical trials (Wei et al. (1995), *Nature* 373:117-122; Ho et al. (1995), *Nature* 373:123-126). Combination therapies including HIVPr inhibitors have offered the best results so far to control AIDS (Mellors, (1996), *Nat. Med.* 2:274-276).

Rapid progress on the structure and activity of HIVPr has taken place since the discovery that it is an aspartic protease (Toh et al. (1985), *Nature* 315:691-692). These include the identification of the HIVPr genome, expression and purification of recombinant enzyme, total chemical synthesis (Schneider and Kent (1988), *Cell* 54:363-368), crystal structure of HIV-1 protease (Wlodawer et al. (1989), *Science* 245:616-621; Navia et al. (1989), *Nature* 337:615-620; Lapatto et al. (1989), *Nature* 342:299-302) and HIV-2 protease (Mulichak and Watenpaugh (1993), *J. Biol. Chem.* 268:13103-13109); Tong et al. (1993), *Proc. Natl. Acad. Sci. USA* 90:8387-8391; Chen and Kuo (1994), *J. Biol. Chem.* 270:21433-21436) and many enzymic property and inhibition studies. These results are well documented in reviews (Debouck and Metcalf (1990), *Drug Devel. Res.* 21:1-17; Tomaselli et al. (1991), *Chimicaoggi-Chemistry Today* 9:6-27; Graves (1991), *Adv. Exp. Med. Biol.* 306:395-405; Wlodawer and Erickson (1993), *Science* 245:616-621); and a book (Kuo (1994), *Methods in Enzymology* Vol. 241).

The active HIVPr is a homodimer of 99-residue monomers. The active-site cleft is located between two monomers with two Asp^{25} residues forming the catalytic apparatus. The active-site cleft is covered by two flaps and can accommodate eight substrate residues. The specificity of the enzyme is somewhat broad (Poorman et al. (1991), *J. Biol. Chem.* 266:14554-14561) which is consistent with the sequence differences of the

eight natural processing sites. An unique specificity of HIVPr is the ability to cleave an X-Pro bond, which appears to be related to the mobility of the active site of the enzyme (Hong et al. (1998), *Protein Sci.* 7:300-305). The specificity of the subsite pockets is also influenced by the side chains bound in adjacent pockets (Ridky et al. (1996), *J. Biol. Chem.* 271:4709-4717).

Each of the four commercial HIVPr inhibitor contains an isostere – $CH(OH)-CH_2-$ which mimics the transition state in the catalytic mechanism of aspartic proteases (Marciniszyn et al., 1976), *J. Biol. Chem.* 251:7088-7094) thereby rendering the tight binding properties of the inhibitor. The position of the isostere, which is equivalent to that of the scissiled bond in the substrate, defines the subsite binding for the inhibitor residues. An example can be seen in a non-commercial inhibitor U-85548 in Figure 1a. The commercial inhibitor drugs, which require good pharmacokinetic properties and high potency, are typically shorter and have less well defined residue boundaries (Figures 1b-d). The interaction of subsite residues in HIVPr with the inhibitors are generally known from the crystal structures of the HIVPr-inhibitor complexes (Wlodawer and Erickson, 1993). The ability of HIVPr inhibitors to suppress HIV replication has been demonstrated in tissue culture and in clinical trials (Wei et al., 1995; Ho et al., 1995). The use of HIVPr inhibitors along with other drugs in combination therapy has offered the best results so far in suppressing HIV propagation *in vivo* (Mellors, 1996).

The first transition-state analogue of aspartic proteases discovered was pepstatin by Marciniszyn et al. (1976). In this study, the hydroxyethylene group, $–CH(OH)-CH_2-$, was identified to mimic the transition state of catalysis with two carbons in tetrahedral conformation, as contrast to the planar conformation of the peptide bond in the substrate. It was concluded that the potency of pepstatin inhibition was related to the presence of this transition-state mimicry in this (isostere) structure. Because the aspartic proteases share common active-site structure and catalytic mechanism, the transition-state isosteres are applicable to all enzymes of this family. This principle was used later to design inhibitors for renin (Szelke,

1985; Boger, 1985) and HIVPr inhibitors (Tomaselli et al., 1991). Other types of isosteres were later designed and shown to be effective in aspartic protease inhibitors. These include, in addition to hydroxyethylene, dihydroxyethylene [-CH(OH)-CH(OH)-], hydroxyethylamine [-CH(OH)-CH_2- NH-], phosphinate [-PO(OH)-CH_2-] and reduced amide [-CH_2-NH-] (Reviewed by Vacca, 1994). In all cases, including the commercial HIVPr inhibitor drugs, a single transition-state isostere is used in an inhibitor since it mimics a substrate peptide with a single hydrolysis site.

The development of resistance to HIVPr inhibitors by the mutation of the HIVPr gene has been clearly demonstrated in *in vitro* experiments (reviews: Mellors et al. (1994), *Nat. Med.* 2:760-765; Ridky and Leis (1995), *J. Biol. Chem.* 271:4709-4717) and in clinical trials (Wei et al. (1995), *Nature* 373:117-122; Ho et al. (1995), *Nature* 373:123-126; Jacobsen et al. (1996), *J. Infect. Diseases* 173:1279-1389; Condra et al. (1996), *J. Viol.* 70:8270-8276; Molla et al. (1996) *Nat. Med.* 2:760-765). The *in vitro* selection of resistant mutants typically takes many passages of HIV in cell culture with increasing inhibitor concentrations for each passage. In patients, the resistance occurs within weeks, owing to the fast replication of virus and fast turnover of the CD4+ T-cells (Coffin, (1995), *Science* 267:483-489).

Comment: U-85548 is an HIV protease inhibitor, but it is not marketed as anti-HIV drug. We use U-85548 here to illustrate the principle of HIV Protease inhibitor design. The other 3 (1b, 1c, 1d) are drugs.

Summary of the Invention

Protease inhibitors, especially viral protease inhibitors such as HIVPr inhibitors, which are effective against drug resistance resulting from the mutations in the protease gene have been developed. These compounds contain two or more isosteres - CH(OH)-CH_2 – which mimic the transition state in the catalytic mechanism of the protease. Design and testing of the inhibitors containing two or more isosters is demonstrated using an HIVPr inhibitor. Unlike known commercial HIVPr inhibitors, these inhibitors do not contain only one isoster having a single orientation which binds to the

HIVPr active site at only one mode. These HIVPr inhibitors bind to HIVPr in two or more modes. They not only bind to the protease active site more tightly, but exhibit significantly better activity against HIVPr-resistant mutants and are less prone to development of resistance.

Brief Description of the Drawings

Figures 1a-1d are formulas of U-85548 (Figure 1a) and known HIVPr inhibitors which have been clinically marketed; Saquinaur or R031-8959 (Figure 1b); Indinavir or L-735,525 (Figure 1c); and Ritonavor ABT-538 (Figure 1d).

Figure 2 is a schematic of UIC 98-056.

Figure 3 is a schematic of the synthesis of UIC-98-056.

Figure 4 is a graph of the relative K_i values of three commercial HIVPr inhibitor drugs, saquinavir, indinavir and ritonavir, and inhibitor UIC-98-056 (using the K_i of wild-type HIVPr as control value = 1) against eleven HIVPr activities: wild-type, and ten HIVPr resistant mutants K20R, M461, L101, G48V, V82A, I64V, I90L, I15V, 184V, and L90M. The K_i values of the wild-type HIVPr are shown in Table II. The relative K_i values (in parenthesis of Table II and in Fig. 4) of the ten resistant mutants were calculated from the ratio of K_i of the mutant HIVPr/K_i of the wild-type HIVPr. The key to the numbering of the mutants is shown in the inset of Fig. 4.

Detailed Description of the Invention

The design and testing of these protease inhibitors is exemplified using HIVPr inhibitors. It is understood, however, that this concept is generally applicable to protease inhibitors, especially aspartic acid protease inhibitors.

Design of HIVPr Inhibitors Effective Against Drug Resistant Mutants

The large body of data on HIVPr resistant mutants selected in the presence of inhibitors *in vitro* and *in vivo* can be summarized as follow:

(a) The resistant mutants observed *in vitro* and *in vivo* appear in both systems at high frequencies.

(b) Many mutations unrelated to resistance are observed in both systems. Additional tests using enzyme inhibition and inhibitor tolerance by HIV mutants are able to clearly establish the resistant mutants.

(c) Resistant mutations differ with inhibitors (Rose et al. (1996), *Proc. Natl. Acad. Sci.*, 93:1648-1653).

(d) Mutants selected from a patient with resistance often, but not always, cross resist to other inhibitors (Gulnik et al. (1995), *Biochemistry*, 34:9282-9287; Rose et al. (1996), *Proc. Natl. Acad. Sci. USA 93*:1648-1653; Condra et al. (1996), *J. Viol.*, 70:8270-8276; Molla et al. (1996), Nat. Med. 2:760-765).

(e) *In vivo* selections produced a consistent and ordered pattern of time-dependent increase of mutation sites per protease molecule (Condra et al. (1995), *Nature* 374:469-471; Jacobsen et al. (1996), *Virology* 206-527-534; Condra et al. (1996), *J. Viol.* 70:8270-8276; Molla et al. (1996), *Nat. Med.* 2:760-765. Increased number of mutation sites correlates with less sensitivity to inhibitors. These studies clearly demonstrated that mutation of HIVPr is responsible for the resistance.

Emerging from clinical resistance studies is a group of about 15 mutation sites on HIVPr (Table I) which account for the resistance of the protease inhibitor drugs, saquinavir, indinavir and ritonavir, and cross resistance of many other inhibitors Rose et al. (1996), *Proc. Natl. Acad. Sci. USA 93*:1648-1653.

Table I: HIV-1 protease resistant mutants compiled from the result of clinical trials against three commercial HIVPr inhibitor drugs indinavir, ritonavir and saquinavir.

Position	10	15	20	24	36	46	48	54	63	64	71	82	84	90	93
Wild Type	L	I	K	L	M	M	G	I	L	I	A	V	I	L	I
Indinavir	I		M	I		I		V	P	V	V	A	V	M	
	V		R			L		A			T	F			
	R											T			
Ritonavir	I	V	R		I	I		V	P		V	F	V		L
												A			
Saquinavir	I				I		V	V	P			I	V	M	
	V								A						

Residues are indicated by single-letter amino acid codes, where A-Ala, F=Phe, G=Gly, I=Ile, K-Lys, L=Leu, M=Met, P=Pro, R=Arg, T=Thr and V=Val*In Vitro* Demonstration of Resistance to Inhibition by HIVPr Mutants.

It is now well accepted that for an HIVPr mutant to prosper in the presence of an inhibitor drug, the mutant enzyme must retain sufficient catalytic activity, which can be expressed in k_{cat}/K_m, with reduced sensitivity to the inhibitor (with increased K_i) (Ermolieff et al., 1997), *Biochemistry* 36:12364-12370). The most complete kinetic model developed to determine the activity of HIVPr (wild type or mutants) was described by Tang and Hartsuck (1995), *FEBS. Lett.* 367:112-116). In this model, the processing activity of wild-type and mutants HIVPr , α, at a given inhibitor concentration, [I], is calculated from kinetic parameters k_{cat}, K_m and K_i based on the equation

$$\alpha = \sigma \{ (k_{cat}/K_m) / [1 + ([I]/K_i)] \} \text{ where } \sigma \text{ is a constant.}$$

This model has been shown to agree with the clinical resistance data. Since the values of k_{cat}/K_m are almost always lower in resistant mutants than in the wild-type HIVPr, it is not suited as a criteria for *in vitro* evaluation of resistance. On the other hand, the K_i values of different known resistant mutants have good correlation to clinical resistance and can be used to indicate if resistance is taking place against an inhibitor.

Structural Changes from the Wild-type to Resistant HIVPr's.

The resistant mutants of HIVPr inhibitors have three dimensional structural changes from that of the wild-type enzyme. The x-ray crystal structures of several resistant mutants of HIVPr have been studied (Chen et al., 1995, *J. Biol.Chem.* 270:21433-21436; Baldwin et al., 1995, *Nature Struct. Biol.* 2:244-249; Kervinen et al., 1996, *Protein & Peptide Lett.* 6:399-406; Hong et al., 1996 & 1997, *Biochemistry* 35:123-126, *Structure and Function of Aspartic Proteases: Retroviral and Cellular Enzymes* (M.N.G. James, ed.). The structural basis underlying the mutation/resistance have also been analyzed by comparing the structures of wild-type and mutant HIVPr complexed to the same inhibitors. Although not all the structural factors involved in resistance is understood at the present, it is known that

one of the most frequent structural changes as a consequences of resistant mutation of HIVPr is the change of the subsite side chain pockets of the enzyme which causes the inhibitor to bind less effectively. Some of the resistant mutation sites are not located in the subsite pockets. However, due to the flexibility of HIVPr conformation (Ridky et al., 1996), *J. Biol. Chem.* 271:4709-4717), the change of subsite pocket conformation can be induced from a distance.

Design and Test of HIVPr Inhibitors that are less vulnerable to resistance

Design principles of current inhibitors which are vulnerable to resistance

To date, all HIVPr inhibitors tested produced HIV resistance in clinical trials because of viral mutations and selection. The main reason for such uniformity is that these inhibitor drugs are designed by the same principle as follows:

In all these inhibitors, an isostere is placed in the polypeptide backbone (or equivalent) of the inhibitor to mark the position of the scissile peptide bond and to mimic the transition state. As illustrated in two examples below, the position of the isostere (as shown by *) defines the assignments of the side chains (A to F) to different subsites of the enzyme. (There are eight subsites). By convention, the substrate subsites on the amino-terminal side of the scissile bond are P_1, P_2, P_3 and P_4 in that order, and the substrate subsites on the carboxyl-terminal side of the scissile bond are P_1', P_2', P_3' and P_4'. The 8 corresponding subsite binding pockets in the enzyme are named S_1 (for binding P_1), S_2 (for binding P_2) . . . . and so on.

Inhibitor 1:

Sequence	A	B	*	C	D	E	F
Subsites	P_2	P_1		P_1'	P_2'	P_3'	P_4'

Inhibitor 2:

Sequence	A	B	C	D	*	E	F
Subsites	P_4	P_3	P_2	P_1		P_1'	P_2'

Even though the residues (A-F) and their sequences in these two inhibitors are the same, the individual residues (A to F) bind different

subsites of the enzyme for two inhibitors because the position of the isostere is different.

The side chains of the inhibitor are designed to fill the subsite pockets of HIVPr, thus creating a tight binding.

It is clear from the in vitro and clinical studies that HIV-1 mutation/resistance can defeat any structure generated by this principle.

Design principle for HIVPr inhibitors that can withstand mutation-resistance.

A new principle for the design of HIVPr inhibitors less vulnerable to mutation-resistance has been developed. The principle is based on placing two isosteres in a single inhibitor. In such an inhibitor, as illustrated in the example, inhibitor 3, below, each residue (A to F) has two subsite-assignments:

Inhibitor 3:

Sequence	A	B	*	C	D	*	E	F	
Subsites	P_2	P_1		P_1'	P_2'		P_3'	P_4'	using left isostere
Subsites	P_4	P_3		P_2	P_1		P_1'	P_2'	using right isostere

For example, depending upon which of the two isosteres is used to bind HIVPr, the residue A can be in either P_2 or P_4 and residue E can bind in either P_3' or P_1' and so on.

The theoretical ability of a two-isostere inhibitor to fight against mutation-resistance of HIV is illustrated in the following example. A resistant mutation of HIVPr in subsite binding pocket S_2 against inhibitor 2 (as described above) would need only to reduce the affinity against residue C. In inhibitor 3, however, the same mutation would need to reduce the affinity of both residues A and C. This is a much more difficult task, especially when residues A and D are structurally different. Even if resistance to 2 residues can be done by multiple mutations in the same subsite, the resulting mutant HIVPr will likely have much less catalytic activity (Ermolieff et al., 1997), thus rendering the mutant strain HIV ineffective.

There is also a kinetic benefit for the two-isostere inhibitors over the one-isostere inhibitors. The two binding modes of a two-isostere inhibitor to HIV protease are represented by the following two equations:

$$E_{free} + I_{free} = EI_1 \quad (1)$$

$$E_{free} = I_{free} = EI_2 \quad (2)$$

where E_{free} and I_{free} are unbound enzyme and inhibitor respectively. EI_1 and EI_2 are inhibitor bound to enzyme by a first isotere and by the second isostere, respectively. The dissociation constants, or inhibition constants, of the equations (1) and (2) and $K_{i,1}$ and $K_{i,2}$ respectively. The overall inhibition constant, K_i, of a two-isostere inhibitor for the enzyme is

$$K_i = (K_{i,1} \times K_{i,2}) / (K_{i,1} + K_{i,2}) \quad (3)$$

The kinetic benefit of a two-isostere inhibitor can be seen in following examples.

(a) Assuming $K_{i,1}$ and $K_{i,2}$ both to be 1×10^{-9} M, based on equation (3), the overall inhibition constant, K_i, is 0.5×10^{-9} M, lower than either $K_{i,1}$ or $K_{i,2}$.

(b) Assuming $K_{i,1}$ and $K_{i,2}$ both to be 1×10^{-9} M and K_i to be 0.5×10^{-9} M for the wild-type HIV protease, and assuming Ki,2 increases 10-fold to 1×10^{-8} M against a resistant mutant of HIV protease, the overall inhibition constant, K_i, for the resistant mutant is 0.9×10^{-9} M, a less than two-fold increase over that of the wild-type enzyme.

Thus, the kinetic benefit based on equation (3) will not only lower the K_i of the inhibitor, but also resist the K_i increase (decrease binding intensity) by resistant mutations.

Applicability to Development of other Inhibitors

Although described herein with specific reference to design of HIVPr inhibitors, it is readily apparent that this concept is generally applicable to the development of effective therapeutics which are targeted against other proteases, especially those aspartic proteases of viral origin.

For example, human cathespin D is involved in breast cancer metastasis (Rochefort (1990) *Semin. Cancer Biol.* 1: 153-160) and in the development of Alzheimer disease in the brain (Siman et al. (1993) *J. Biol.*

Chem. 268: 16602-16609). The design of transition-state inhibitors for cathespin D to control these diseases has been attempted (Majer et al. (1997) *Protein Sci.* 6: 1458-1466). Human renin, an aspartic protease, is the target for inhibitor design of isostere-containing transition-state inhibitors for the control of hypertension (Hoover et al. (1995) *Adv. Exptl. Med. Biol.* 362: 167-180. There are a number of examples of proteases in pathogens. For example, malaria causing protozoa *Plasmodium* contains two aspartic proteases, plasmepsin I and II, which are also targets for transition-state inhibitor drugs (Carroll et al. (1998) *Bioorg. Med. Chem.* 8: 2315-2320; Carroll et al. (1998) *Bioorg. Med. Lett.* 8: 3203-3206). Retroviruses, which cause in addition to immunodeficiency and leukemia in human and animals and different tumors, contain aspartic proteases with processing functions similar to that of HIV protease (Weiss et al. (1984) *RNA Tumor Viruses, Molecular biology of Tumor Viruses*, Second Edition, Vol. 1,Cold Spring Harbor, NY). These proteases are all drug design targets for the control of diseases. The human genome also contains an endogenous virus which expresses active aspartic protease, which has been studied for inhibition by transition-state, isostere-containing inhibitors (Towler et al. (1998) *Biochemistry* 37: 17137-17144). Drugs targeted to these protease can benefit from the design utilizing two or more isosteres in a single inhibitor molecule in order to enhance the potency and withstand development of resistance. Examples of other isosteres which mimic the transistion state of aspartic protease catalysis are shown by Vacca, "Design of Tight-Binding Human Immunodeficiency Virus Type 1 Protease Inhibitors", Methods in Enzymology, 241, 313-333 (1994).

Pharmaceutical Compositions

The protease inhibitors described herein are administered to a patient in need of treatment, or prophylactically, using methods and formulations similar to those for other HIVPr inhibitors. The protease inhibitor is preferably administered orally. In the case of HIVPr inhibitors, the protease inhibitor is most preferably administered as part of a "cocktail" including other anti-HIV compounds such as the nucleosides like AZT. The most

recent guideline for such therapy by the International AIDS Society is described in Carpenter, Fischel, Hammer et al. (1998) *J. Am. Med. Assoc.* 280: 78-86. The regimens and the choice of drug combinations are dependent on the resistance genotype and phenotype of the HIV strains. The therapeutic strategy is summarized by Larder, Richman and Vella (1998) HIV Resistance and Implications for Therapy, MediCom.

This process is demonstrated by the following non-limiting example.

Example 1: Design and Synthesis of Two-isostere HIVPr Inhibitor UIC-98-056.

Based on the principle described above and other considerations, HIVPr inhibitor UIC-98-056 was designed and synthesized. The structure of this inhibitor is shown in Figure 2.

Synthesis of HIV Protease Inhibitor UIC-98-056:

The synthesis of HIV protease inhibitor UIC-98-056 with hydroxyethylene and hydroxyethylamine isosteres is outlined in Figure 3. The known lactone **1** was converted to acid **2** by lithium hydroxide mediated hydrolysis followed by protection of the alcohol functionality as *tert*-butyldimethylsilyl ether (Ghosh et al., 1998, *Synthesis*, 937 (Review); Ghosh et al., 1991, *J. Org. Chem.* 56:6500; Evens et al., 1985). The previously described (Ghosh et al., 1992, *J. Chem. Soc., Chem. Co.*, 273; Ghosh et al., 1998, *Synthesis*, 937 (Review)) azido epoxide **3** was reacted with isobutylamine in 2-propanol at 80°C for 4 h and the resulting azidoalcohol was treated with *m*-tetrahydropyranyloxybenzenesulfonyl chloride **4** (Metanilic acid was diazotized at 0°C and the resulting salt was boiled with water to obtain 3-hydroxybenzene sulfonic acid which was then treated with thionyl chloride/catalytic DMF/reflux to obtain sulfonyl chloride. The hydroxy group of the resulting 3-hydroxybenzene sulfonyl chloride was protected as THP ether by treating with DHP/catalytic PPTS in methylene chloride to get **4** as an oil.) in the presence of aqueous $NaHCO_3$ to provide the sulfonamide derivative **5**. The azide functionality of **5** was hydrogenated over 10% Pd-C in methanol to afford the corresponding amine which was coupled with the acid **2** in the presence of 1-[3-dimethylaminopropyl]-3-

ethylcarodiimide (EDC) and 1-hydroxybenzotriazole (HOBt) to afford the amide **6**. Removal of the BOC group by treatment with aqueous hydrochloric acid and alkoxycarbonylation of the resulting amine with the known (Ghosh et al., 1993a) mixed active carbonate **7** in methylene chloride in the presence of 3 equivalents of triethylamine (Et_3N) 23°C for 12 h afforded the inhibitor **8** (UIC-98-056).

Experimental:

(2*S*,4*S*,5*R*)-5-[N-(*tert*-Butoxycarbonyl)amino-4-*tert*-butyldimethylsilyloxy-2,5 -dibenzyl pentanoic acid (2):

To a stirred suspension of lactone **1** (140 mg, 0.354 mmol) in a mixture (1:1) of DME and water (3 mL) at 0°C was added LiOH monohydrate (29 mg, 0.71 mmol). After being stirred for 2h, the reaction mixture was concentrated under reduced pressure. The aqueous layer was extracted with ethyl acetate (EtOAc) (2 x 10 mL). The combined organic layer was dried over anhydrous Na_2SO_4 and then concentrated under reduced pressure. The crude acid was dried under vacuum and dissolved in DMF (5 mL). Imidazole (956 mg, 6.4 mmol) was then added in portions followed by TBSCl (1.33 g, 12.7 mmol). The resulting reaction mixture was stirred at 23°C for 48 h and then diluted with EtOAc (25 mL). The organic layer was washed with brine (3 x 20 mL) and dried over anhydrous Na_2SO_4. Evaporation of the solvent gave crude acid which was chromatographed (50% EtOAc/hexanes as the eluent) over silica gel to provide the title acid **2** (125 mg, 72%) as an oil: ^{1}H NMR exhibits a mixture of rotational isomers: ^{1}H NMR ($CDCl_3$, 400 Mhz): δ, 7.28-7.06 (m, 10H), 6.28 (m) and 4.72 (d, J=9.4 Hz) (for 1H), 4.11-3.64 (m, 2H), 2.99-2.46 (m, 5H), 1.88 (m) and 1.58 (m) (for 2H), 1.33 (s, 9H), 0.96 (s, 9H), 0.072-0.056 (m, 6H); IR (neat): 2952, 2928, 2858, 1711, 1658, 1406 cm^{-1}.

(2*R*,3*S*)-3-Azido-2-hydroxy-1-[[N-isobutylamino-N'-[(3-tetrahydropyranoxy)-benzenesulfonamido]yl]-4-phenylbutane (5):

To a stirred solution of **3** (546 mg, 2.9 mmol) in isopropanol (7 mL) was added isobtylamine (426 mg, 5.76 mmol) and the resulting reaction mixture was heated at 75°C for 4 h. After this period, solvents were

evaporated under reduced pressure and the resulting amine was dried under vacuum. To a stirred solution of this amine in CH_2Cl_2 (7 mL) was added sulfonyl chloride **4** (794 mg, 2.9 mmol) followed by aqueous $NaHCO_3$ solution (10%, 7 mL). The resulting mixture was stirred at 23°C for 12 h. After this period, the organic layer was separated and then dried over anhydrous Na_2SO_4. Evaporation of the solvent under reduced pressure and chromatography of the residue over silica gel (25-30% EtOAc/hexanes) afforded the sulfonamide **5** (1.029 g, 85%) as an oil: ^1H NMR ($CDCl_3$, 200 Mhz): δ, 7.48-7.21 (m, 9H), 5.48 (m, 1H), 3.83-3.62 (m, 2H), 3.59-3.47 (m, 2H), 3.53 (dd, 1H, J=12.4, 3.3 Hz), 2.26 (dd, 1H, J=12.4, 8.8 Hz), 3.16-3.02 (m, 3H), 3.0-2.72 (m, 2H), 1.9-1.63 (m, 7H), 0.89 (dd, 6H, J=11, 6.6 Hz).

(2*R*,2'*R*,3*S*,4'*S*,5'*S*)-3-[N-[2'-Benzyl-5'-[(*tert*-butoxycarbonyl)amino]-4'-*tert*-butyldimethylsilyloxy-6'phenyl-1'-oxo]hexyl]amino-2-hydroxy-1-[N-isobutyl-amino-N'[3-tetrahydropyranoxybenzene)sulfonamido]]-4-phenylbutane (6):

To a stirred solution of the azide **5** (139 mg, 0.33 mmol) in methanol (3 mL) at 23°C was suspended palladium on charcoal (10%, 15 mg). The resulting mixture was hydrogenated under a balloon filled hydrogen atmosphere for 12 h. After this period, the catalyst was filtered off through a pad of celite and the filter cake was washed with ethyl acetate (5 mL). Evaporation of the solvent furnished the amine obtained which was used for next reaction without further purification.

To a stirred solution of the acid **2** (120 mg, 0.24 mmol) in a mixture (1:3) of DMF and CH_2Cl_2 (3 mL) at 0°C were added HOBt (32 mg, 0.24 mmol) and EDC (46 mg, 0.24 mmol). The resulting mixture was stirred at 0°C for 10 min. After this period, the above crude amine (156 mg, 0.4 mmol) in CH_2Cl_2 (0.5 ml) followed by diisopropylethylamine (51 mg, 0.4 mmol) were added. The resulting reaction mixture was stirred at 23°C for 12 h. After this period, CH_2Cl_2 (15 mL) was added and the organic layer was washed with brine (2 x 10 mL) and then dried over anhydrous Na_2SO_4. Evaporation of the solvent under reduced pressure afforded a residue which was chromatographed (50% EtOAc/hexanes as the eluent) to obtain the

amide **6** (221 mg, 68%) as an oil: ^{1}H NMR ($CDCl_3$, 400 MHz) δ, 7.43-6.9 (m, 19H), 5.82 (m, 1H), 5.48 (m, 1H), 4.73 (d, 1H, J=13.4 Hz), 3.93-3.82 (m, 3H), 3.65-3.51 (m, 2H), 3.15-2.21 (m, 13H), 1.90-1.63 (m, 9H), 1.38 (s, 9H), 0.93 (s, 9H), 0.78 (q, 6H, J=6.5 Hz), 0.02 (s, 3H), 0.011 (s, 3H).

(1'*S*,2*R*,2'*R*,3*S*,5'*S*)-3-[N-[2'-Benzyl-5'-[N-(2-tetrahydrofuranyloxycarbonyl]amino-4'-hydroxy-6'-phenyl-1'-oxo]hexyl]amino-2-hydroxy-1-[N-isobutylamino-N'[3-hydroxybenzene)sulfonamido]]-4-[phenylbutane (8):

To a stirred solution of the BOC derivative **6** (98 mg, 0.1 mmol) in a mixture (1:1) of EtOAc and water (2 mL) at 0°C was added concentrated hydrochloric acid (0.2 mL). The resulting mixture was stirred for 24 h and the reaction was concentrated under reduced pressure. The residue was dried under vacuum for 1 h and then dissolved in CH_2Cl_2 (2 mL). A solution of mixed carbonate **7** (29 mg, 0.1 mmol) in CH_2Cl_2 (1 mL) followed by Et_3N (9.9 mg, 0.1 mmol) were added. The mixture was stirred for 12 h and then it was diluted with CH_2Cl_2 (15 mL) and washed with brine (10 mL). The organic layer was dried over anhydrous Na_2SO_4. Evaporation of the solvent under reduced pressure gave a residue which was chromatographed over silica gel (80% EtOAc/hexanes as the eluent) to furnish the inhibitor **8** (35 mg, 45%): ^{1}H NMR ($CDCl_3$, 200 Mhz) δ, 7.64-7.03 (m, 19H), 5.64 (d, 1H, J=8.1 Hz), 5.29 (s, 2H), 5.1 (m, 1H), 4.74 (d, 1H, J=8.4 Hz), 4.22-3.58 (m, 8H), 3.04-2.32 (m, 8H), 2.15-1.62 (m, 8H), 0.9 (d, 6H, J=6.3 Hz).

Example 2: Demonstration that Inhibitor UIC-98-056 Can Withstand Resistance.

The inhibition constant, K_i, of UIC-98-056 was determined for the wild type HIV-1 protease and 10 mutants resistant to HIVPr inhibitors using the methods described by Ermolieff et al. (1997), *Biochemistry* 36:12364-12370. The wild-type HIV-1 Pr was produced as recombinant enzyme in *E. coli* as described by Ido et al. (1991) *J. Biol. Chem.* 266:24359-24366. The mutant enzymes were made by site-directed mutagenesis of the HIVPr gene either as described by Ermolieff et al., 1997, *Biochemistry* 36:12364-12370 or by a similar procedure. These mutants were identified in clinical trials

and *in vitro* studies to resist saquinvair (mutants G48V and L90M, Jacobsen et al., 1996, *J. Infect. Diseases* 173:1379-1387), indinavir (mutants V82A, M46I and L10I, Condra et al., 1995; Lander, Richman and Vella (1998) HIV Resistance and Implications for Therapy MediCom.) and ritonavir (mutants L90M, V82A, K20R and M46I, Molla et al., 1996, *Nat. Med.* 2:760-765; Carder et al. (1998)). For direct comparison, the K_i values against the wild-type HIVPr and ten mutants were also determined for saquinavir, indinavir and ritonavir.

Table II shows these K_i values and the ratios (in parenthesis) between the inhibition constant of the mutants, $K_{i,mut}$, to the inhibition constant of the wild-type HIVPr taken as 1.0. The latter results are also plotted in Figure 3. It can be seen clearly that for three commercial drugs, the $K_{i,mut}$ values of the resistant mutants are consistently higher than K_i of the wild-type HIVPr (Table II and Figure 3), indicating the resistance and cross-resistance properties of the mutants. In contrast, the same comparison for inhibitor UIC-98-056, the $K_{i,mut}$ values are nearly the same as K_i value of the wild-type HIVPr for all mutants except mutant I84V, which increased 9-fold. Even in this mutant, the only significant increase observed is much less than the K_i increase of the other three commercial inhibitors, which are 22-fold for ritonavir, 18-fold for indinavir and 13-fold for sequinavir (Table II and Figure 3).

Table II: K_i values of the wild-type and resistant mutants of HIV-1 protease. Data are reported with standard errors and the fold of K_i increase from that of the wild-type are shown in parenthesis.

K_i (nM)				
	Saquinavir	**Indinavir**	**Ritonavir**	**UIC-98-056**
W.T.	0.20±0.06	0.10±0.02	0.20±0.10	6.20±0.80
V82A	1.53±0.54(7.7)	5.18±0.55(51.8)	2.40±0.37(12.0)	6.23±1.20(1.0)
I84V	2.62±0.27(13.1)	1.81±0.03(18.1)	4.49±1.73(22.5)	55.30±0.46(8.9)
I15V	0.38±0.19(1.9)	0.76±0.13(7.6)	0.78±0.16(3.9)	1.22±0.14(0.2)
K20R	0.81±0.35(4.1)	3.16±0.61(31.6)	1.99±0.60(10.0)	2.90±0.74(0.5)
M46I	3.36±0.52(16.8)	1.01±0.49(10.1)	14.73±4.60(73.7)	3.16±0.81(0.5)
L10I	1.74±0.20(8.7)	0.52±0.23(5.2)	1.50±0.26(7.5)	1.03±0.25(0.2)
G48V	2.70±0.20(13.5)	0.26±0.02(2.6)	0.10±0.03(0.5)	8.70±1.40(1.4)
L90M	0.60±0.04(3.0)	0.48±0.06(4.8)	0.51±0.06(2.6)	2.10±0.50(0.3)
I64V	0.94±0.55(4.7)	2.88±0.60(28.8)	2.50±0.66(12.5)	6.24±1,52(1.0)
I93L	6.82±1.78(34.1)	2.83±0.76(28.3)	1.20±0.52(6.0)	3.05±0.64(0.5)

These ten mutants are representative resistant mutants in the clinical trials for three commercial HIVPr inhibitor drugs. The results in Table II and Figure 3 confirm the resistance by the observation of the K_i increases from the wild-type to the mutant HIVPr against three commercial inhibitors. There are also considerable cross resistance of these mutants against all three commercial drugs (Table II and Figure 3) as already well known in the literature (Rose et al., 1996, *Proc. Natl. Acad. Sci.* USA 93:1648-1653; Winslow and Otto, 1995, *AIDS* 9 (suppl A):S183-S192). Nine out of ten of the K_i values of inhibitor UIC-98-056 did not change from the wild-type to mutant HIVPr's. The single K_1 increase for I84V is also less significant than that of the three commercial drugs. This property, which is unique and has not been previously accomplished, indicates that UCI-98-056 can withstand the development of HIVPr mutation-resistance.

We claim:

1. A protease inhibitor comprising two or more transition-state isosteres.
2. The inhibitor of claim 1 wherein the transition-state isostere is -CH(OH)-CH_2-.
3. The inhibitor of claim 1 wherein the protease inhibitor inhibits an aspartic acid protease.
4. The inhibitor of claim 3 wherein the protease inhibitor inhibits HIV protease.
5. The inhibitor of claim 1 which is UIC-98-056.
6. The inhibitor of claim 2 wherein the CH(OH)-CH_2 is substituted with two other kinds of isosteres.
7. A method for treating a patient infected with a pathogen expressing a protease comprising administering a protease inhibitor comprising two or more transition-state isosteres.
8. The method of claim 7 wherein the transition-state isostere is - CH(OH)-CH_2-.
9. The method of claim 7 wherein the protease inhibitor inhibits an aspartic acid protease.
10. The method of claim 9 wherein the protease inhibitor inhibits HIV protease.
11. The method of claim 10 wherein the inhibitor is UIC-98-056.
12. The method of claim 8 wherein the CH(OH)-CH_2 is substituted with two other kinds of isosteres.

PROTEASE INHIBITORS THAT OVERCOME DRUG RESISTANCE

Abstract of the Invention

HIV protease inhibitors are among the most powerful drugs in suppressing HIV in human patients. However, HIV developed resistance to all protease inhibitor drugs so far marketed or used in clinical trials. HIV generates resistance by mutating its protease. The strains of HIV containing mutant proteases less vulnerable to inhibitor drug are able to replicate better and maintain the infection. No effective principle exists for the design of resistance-proof HIV protease inhibitors (HIVPr). A new inhibitor has been developed based on a new concept for designing resistance invulnerable HIVPr inhibitors. *In vitro* data have shown that this inhibitor is effective against many known HIVPr mutants resistant to other HIVPr inhibitor drugs. The new concept is, therefore, generally applicable for the design of other resistance invulnerable HIVPr inhibitor drugs.

Chem. 268: 16602-16609). The design of transition-state inhibitors for cathespin D to control these diseases has been attempted (Majer et al. (1997) *Protein Sci.* 6: 1458-1466). Human renin, an aspartic protease, is the target for inhibitor design of isostere-containing transition-state inhibitors for the control of hypertension (Hoover et al. (1995) *Adv. Exptl. Med. Biol.* 362: 167-180. There are a number of examples of proteases in pathogens. For example, malaria causing protozoa *Plasmodium* contains two aspartic proteases, plasmepsin I and II, which are also targets for transition-state inhibitor drugs (Carroll et al. (1998) *Bioorg. Med. Chem.* 8: 2315-2320; Carroll et al. (1998) *Bioorg. Med. Lett.* 8: 3203-3206). Retroviruses, which cause in addition to immunodeficiency and leukemia in human and animals and different tumors, contain aspartic proteases with processing functions similar to that of HIV protease (Weiss et al. (1984) *RNA Tumor Viruses, Molecular biology of Tumor Viruses*, Second Edition, Vol. 1,Cold Spring Harbor, NY). These proteases are all drug design targets for the control of diseases. The human genome also contains an endogenous virus which expresses active aspartic protease, which has been studied for inhibition by transition-state, isostere-containing inhibitors (Towler et al. (1998) *Biochemistry* 37: 17137-17144). Drugs targeted to these protease can benefit from the design utilizing two or more isosteres in a single inhibitor molecule in order to enhance the potency and withstand development of resistance. Examples of other isosteres which mimic the transistion state of aspartic protease catalysis are shown by Vacca, "Design of Tight-Binding Human Immunodeficiency Virus Type 1 Protease Inhibitors", Methods in Enzymology, 241, 313-333 (1994), incorporated herein.

Pharmaceutical Compositions

The protease inhibitors described herein are administered to a patient in need of treatment, or prophylactically, using methods and formulations similar to those for other HIVPr inhibitors. The protease inhibitor is preferably administered orally. In the case of HIVPr inhibitors, the protease inhibitor is most preferably administered as part of a "cocktail" including other anti-HIV compounds such as the nucleosides like AZT. The most

FIGURE 1A

U-85548

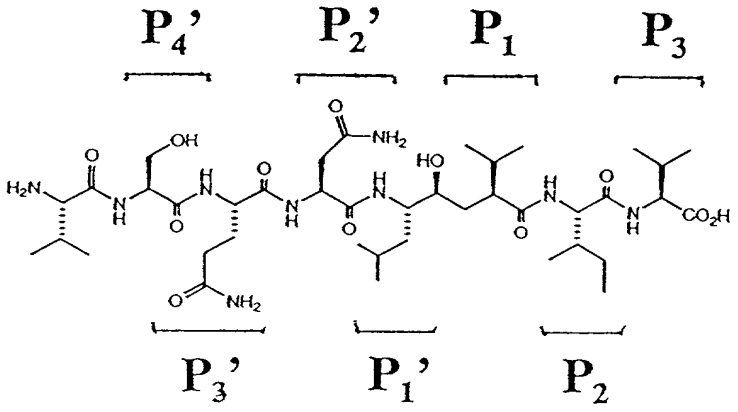


FIGURE 1B

Saquinavir
(Ro31-8959)

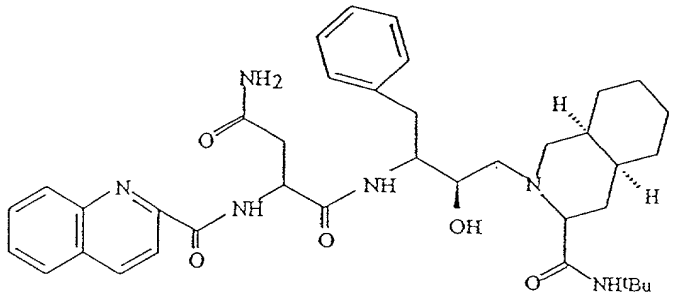


FIGURE 1C

Indinavir
(L-735,524)

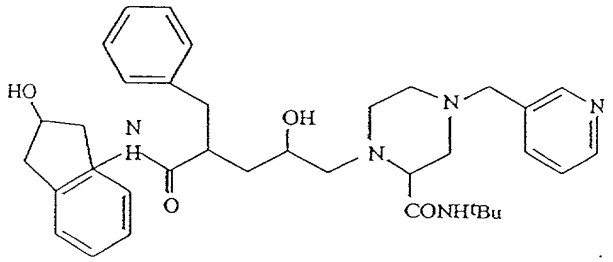


FIGURE 1D

Ritonavir
(ABT-538)

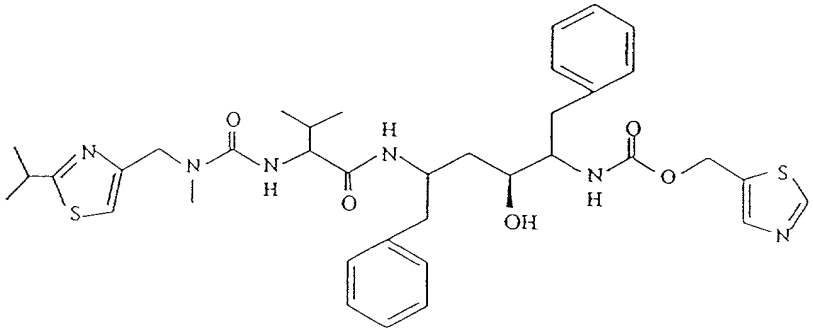
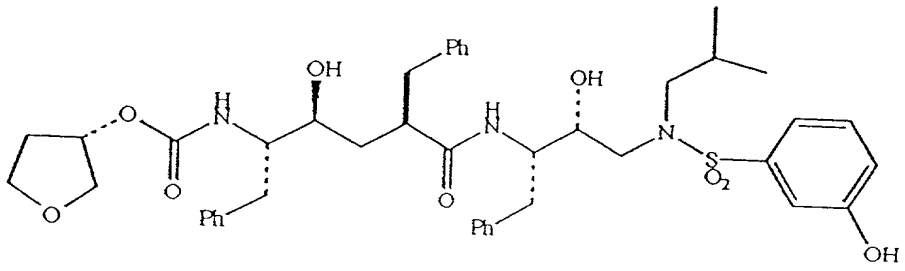


Fig 1

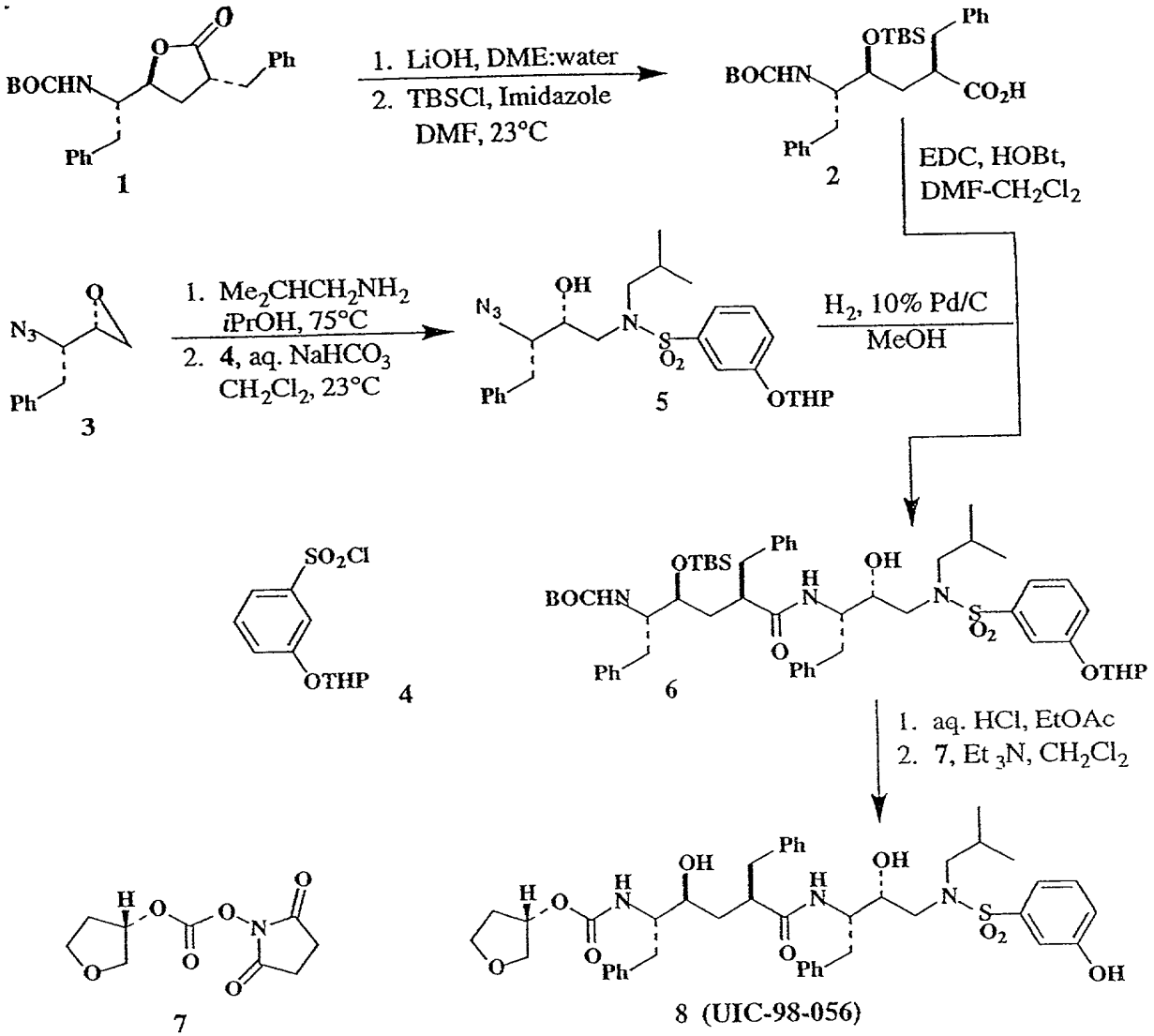


UIC-98-056

Molecular Formula : $C_{44}H_{55}N_3O_9S$

Molecular Weight : **801**

Fig 2



(Figure 3)

Fig 3

DOCKET ALARM

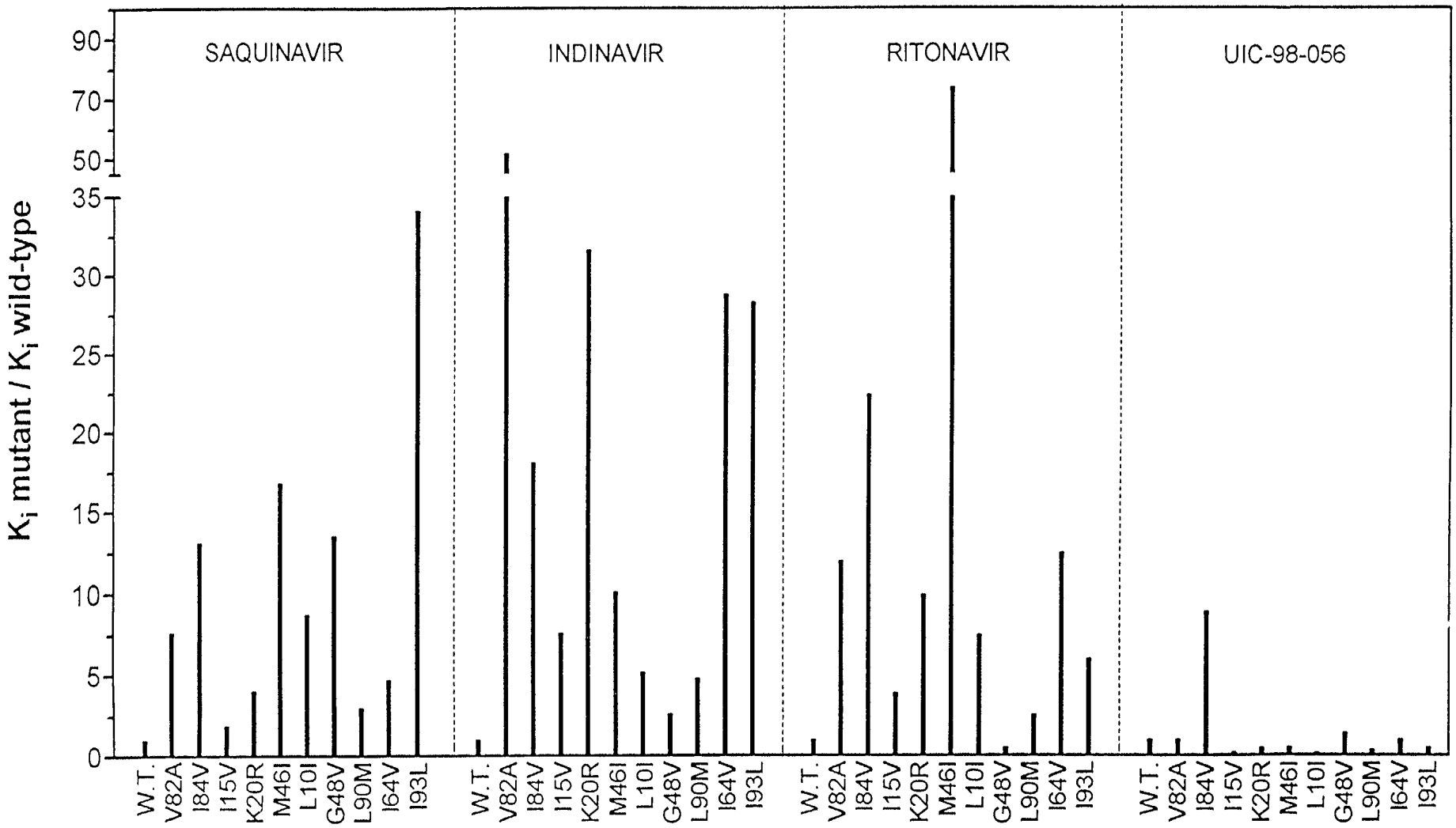


FIGURE 4

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DECLARATION FOR UTILITY OR DESIGN PATENT APPLICATION (37 CFR 1.63)

☒ Declaration Submitted with Initial Filing OR ☐ Declaration Submitted after Initial Filing (surcharge (37 CFR 1.16 (e)) required)

Attorney Docket Number	OMRF 176
First Named Inventor	Jordan J. N. Tang
COMPLETE IF KNOWN	
Application Number	/
Filing Date	February 18, 2000
Group Art Unit	
Examiner Name	

As a below named inventor, I hereby declare that:

My residence, post office address, and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

PROTEASE INHIBITORS THAT OVERCOME DRUG RESISTANCE

(Title of the Invention)

the specification of which

☒ is attached hereto

OR

☐ was filed on (MM/DD/YYYY) [ ] as United States Application Number or PCT International Application Number [ ] and was amended on (MM/DD/YYYY) [ ] (if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment specifically referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR 1.56.

I hereby claim foreign priority benefits under 35 U.S.C. 119(a)-(d) or 365(b) of any foreign application(s) for patent or inventor's certificate, or 365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or of any PCT international application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application Number(s)	Country	Foreign Filing Date (MM/DD/YYYY)	Priority Not Claimed	Certified Copy Attached? YES	NO
			☐	☐	☐
			☐	☐	☐
			☐	☐	☐
			☐	☐	☐

☐ Additional foreign application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto:

I hereby claim the benefit under 35 U.S.C. 119(e) of any United States provisional application(s) listed below.

Application Number(s)	Filing Date (MM/DD/YYYY)	
60/120,835	February 19, 1999	☐ Additional provisional application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto.

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DECLARATION — Utility or Design Patent Application

I hereby claim the benefit under 35 U.S.C. 120 of any United States application(s), or 365(c) of any PCT international application designating the United States of America, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of 35 U.S.C. 112, I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application.

U.S. Parent Application or PCT Parent Number	Parent Filing Date (MM/DD/YYYY)	Parent Patent Number *(if applicable)*

☐ Additional U.S. or PCT international application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto.

As a named inventor, I hereby appoint the following registered practitioner(s) to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith: ☐ Customer Number [ ] → *Place Customer Number Bar Code Label here*

OR

☒ Registered practitioner(s) name/registration number listed below

Name	Registration Number	Name	Registration Number
Patrea L. Pabst	31,284		
Robert A. Hodges	41,074		
Kevin W. King	42,737		

☐ Additional registered practitioner(s) named on supplemental Registered Practitioner Information sheet PTO/SB/02C attached hereto.

Direct all correspondence to: ☐ Customer Number or Bar Code Label [ ] *OR* ☒ Correspondence address below

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Name of Sole or First Inventor: ☐ A petition has been filed for this unsigned inventor

Given Name (first and middle [if any])	Family Name or Surname
Jordan J. N.	Tang

Inventor's Signature						**Date**	
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☐ Additional inventors are being named on the ____supplemental Additional Inventor(s) sheet(s) PTO/SB/02A attached hereto

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PTO/SB/02A (3-97)
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DECLARATION

ADDITIONAL INVENTOR(S)
Supplemental Sheet
Page ___ of ___

Name of Additional Joint Inventor, if any:	☐ A petition has been filed for this unsigned inventor						
Given Name (first and middle [if any])		Family Name or Surname					
Arun K.		Ghosh					
Inventor's Signature						**Date**	
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Name of Additional Joint Inventor, if any:	☐ A petition has been filed for this unsigned inventor						
Given Name (first and middle [if any])		Family Name or Surname					
Inventor's Signature						**Date**	
Residence: City		**State**		**Country**		**Citizenship**	
Post Office Address							
Post Office Address							
City		**State**		**ZIP**		**Country**	

Name of Additional Joint Inventor, if any:	☐ A petition has been filed for this unsigned inventor						
Given Name (first and middle [if any])		Family Name or Surname					
Inventor's Signature						**Date**	
Residence: City		**State**		**Country**		**Citizenship**	
Post Office Address							
Post Office Address							
City		**State**		**ZIP**		**Country**	

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